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14. ABSTRACT
While a strong rationale for chemoprevention of ovarian carcinoma exists, a mechanism for the comprehensive evaluation of novel compounds is severely impeded by the lack of a validated animal model of spontaneous ovarian carcinogenesis. At present, there is no verified, established model for this disease. In rodents, this type of cancer does not spontaneously develop. While studies investigating "induced" carcinomas have been performed they are hindered by biologic differences in induced and spontaneous tumor formation. Identification of spontaneous ovarian carcinogenesis in the laying hen (Gallus Domesticus) may provide the answer to this dilemma. Multiple reports have demonstrated a 30-50% rate of spontaneously arising genital tract adenocarcinomas in hens of 3-6 years of age. Thus, the purpose of this study will be to utilize this animal model to evaluate the ability of a COX-2 inhibitor to reduce the incidence of spontaneous ovarian carcinogenesis in this animal model. More importantly, identification of promising agents in surrogate animal models that simulate a high risk population would significantly impact the strategy of cancer chemoprevention for ovarian carcinoma and lead to subsequent endeavors in this neglected area of study.

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ANNUAL REPORT: CONTROLLED TRIAL OF CHEMOPREVENTION USING COX-2 INHIBITORS IN AN AVIAN MODEL OF SPONTANEOUS OVARIAN CARCINOGENESIS (W81XWH-04-1-0322)

INVESTIGATORS: Mack N. Barnes MD, Wallace D. Berry PhD

INTRODUCTION

Ovarian carcinoma is recognized as an indolent disease process most often detected in the most advanced stages of disease. As a result, little improvement in long term overall survival has been realized through utilization of interventions directed at therapy for Stage II/IV ovarian cancer (1). Therefore, when considering strategies to reduce deaths attributable to ovarian carcinoma, prevention of disease represents the most rational approach. A critical component of developing chemopreventive strategies in ovarian carcinoma is identification of promising new compounds in validated animal models of spontaneous ovarian carcinogenesis. A novel avian model utilizes the laying hen (*Gallus Domesticus*) and exploits the high rate of spontaneous ovarian carcinogenesis observed in this avian species. This model has had demonstrated utility in our prior studies examining the ability of a progestational agent to reduce ovarian cancer incidence (2,3). Therefore, the purpose of the current study (in progress) is to examine the ability of a COX-2 inhibitor to decrease the rate of development of ovarian carcinoma in an avian model of spontaneous ovarian carcinogenesis. If this project demonstrates positive results, the scope of this study will further efforts toward clinical trials in patients at elevated risk of ovarian cancer using NSAID derivatives as chemopreventive agents. Moreover, as this patient group is subject to elevated risk of breast cancer, these clinical efforts will be further bolstered by epidemiological evidence supporting a protective effect of NSAIDs against the development of related breast cancers in this patient population (4).

BODY

As originally proposed, this study entails two specific aims directed at 1) identification of a safe dose of COX-2 inhibitor (Rimadyl) followed by 2) a controlled trial using this agent to determine ability to prevent development of ovarian carcinoma in this animal model. Progress in this study continues along the proposed timeline outlined in the original statement of work. Specifically, aim one has been completed, the large scale controlled trial was initiated on schedule and plans for sacrifice of remaining hens with concurrent histologic assessment for the presence of ovarian carcinoma are in place. Details regarding this work is provided subsequently.

As noted in the original statement of work specific aim#1 was developed to determine the maximally tolerated dose (mg/kg) of COX-2 inhibitor, admixed with standard feed, in the laying hen. The effect of varying doses of COX-2 inhibitor

on egg-laying activity as a surrogate marker for ovulatory frequency were also assessed. This aim was felt to be important, given the lethal effects of high doses of indomethecin observed in laying hens involved in previous studies (personal communication: W. Berry) and the dramatic effect of decreased egg laying activity of Depo-Provera administered to similar laying hens (3). In addition, there is a paucity of information regarding the use of COX-2 inhibitors in the avian hen. Rimadyl (Pharmacia Inc.) is a COX-2 specific inhibitor approved for use in veterinary medicine and available in a stable powder form. Once identified, the maximally tolerated dose would then be used in the controlled trial.

As reported previously upon completion of this portion of work, three dose levels were established as follows:

Table 1

Cohort	Dose of COX-2 Inhibitor
Control:	0 mg/kg body weight
Dose 1:	2.5 mg/kg body weight
Dose 2:	5 mg/kg body weight
Dose 3:	10 mg/kg body weight

Rimadyl was added with other feed ingredients at the time feed was mixed in the feed mill and delivered to the hens. 5 hens were utilized per dose level for a total of 20 hens. The hens were monitored for egg laying activity as defined by percent egg laying activity control (0 mg/kg) and egg shell quality. In addition, toxicity was monitored based on general appearance, feather quality, evidence of gastrointestinal bleeding, and death. Over an 8 week study period (8/04-10/04), no toxicity and no lethal events were noted. In addition, there did not appear to be any effect on ovulatory activity, even at the highest dose level. Given, the ability of these hens to tolerate the highest dose level, 10mg/kg body weight was determined to be the dose we would utilize in the controlled trial. This information remains unchanged from that reported in 2005.

In order to demonstrate the ability of a COX-2 inihibitor to prevent the spontaneous development of genital tract adenocarcinoma in the laying hen a controlled trial was proposed (Specific Aim #2). The plan, as outlined in the statement of work, entailed acquisition of hens, administration of Rimadyl mixed in feed over an 48 month study period (with provision of a non-exposed control group of hens), followed by sacrifice of remaining hens and histologic documentation of presence or absence of adenocarcinoma. This portion of work is also proceeding in accordance with originally proposed timelines.

As outlined in the proposal, a power calculation was performed assuming a 40% incidence of genital tract adenocarcinoma. It was estimated that to identify a reduction in disease of 20% at the 0.05 level of significance a treatment group of 88 hens and a control group of 88 hens would be required. The informative cases

defined as those hens completing the course of treatment (as previously used in a published study. Significant attrition from non-malignant causes is known to occur as hens age. Therefore, to initiate this study 480 hens (*Gallus Domesticus*) were acquired 8/3/04.

Table 2. Specific Information Regarding Origin of Animals

Breed	Gallus Domesticus
Age	2 years
Source	Dr D Roland. Prof Nutrion, Auburn Univ.

This allowed the establishment of a treatment group (240 hens) and a control group (240 hens) that should account for attrition. It is our practice to allow a period of time for hens to "acclimate" and, therefore, a period of 8 weeks was utilized to allow the hens to acclimate. The controlled study was then initiated November 19, 2004, using a daily dose of Rimadyl of 10mg/kg body weight. This trial is ongoing at the date of this report and planned for 48 months. As of 3/1/06, 400 hens were initiated on study 11/19/04 as noted. One hundred and seventy one hens have expired and undergone necropsy to date and these specimens are in storage. Ovarian specimens have been obtained from these hens to determine incidence of ovarian cancer when histologic analysis is completed. Currently, 229 hens remain alive. The current plan is to continue the study medication until late October. Hens that expire will undergo necropsy, while remaining hens in 10/06 will be sacrificed in accordance with the original timeline. Following, sacrifice of all hens the specimens will be reviewed histologically for the presence or absence of cancer to determine the effectiveness of the COX-2 inhibitor to prevent the development of ovarian cancer. (*As of this resubmission, approximately 70 hens in each group remain alive. Ovarian tissues, and suspected malignant tissues have been harvested from each animal and processed in formalin and refrigerated in preparation for transfer to University of Alabama at Birmingham: Personal communication W. Berry)*

Future work will focus on harvesting remaining tissues to facilitate histologic evaluation for the presence or absence of genital tract adenocarcinoma. As noted above, tissues from hens that have expired have been harvested and placed in formalin and refrigerated. In October or 2006, the remainder of the hens will be sacrificed and tissues collected and again placed in formalin. Once all tissues are collected histologic preparation and examination will proceed. William Grizzle PhD and staff will oversee preparation of hematoxylin and eosin (H&E) slides of harvested tissues. Dr Grizzle will provide a "blinded" pathologic evaluation of presence or absence of genital tract adenocarcinoma for each subject. Statistical analysis of these results (in accordance with previously described statistical parameters) will determine if a difference in the incidence of genital tract adenocarcinoma in the treatment group versus the control group is present. These results will be reported in the next Annual Report.

KEY RESEARCH ACCOMPLISHMENTS

- Dose Finding Study completed 10/04
- No toxicity demonstrated for Rimadyl (COX-2 inhibitor) at 10mg/kg body weight and this dose selected for treatment group
- Hens acquired for trial and acclimated (8/04-10/04)
- Controlled trial initiated 11/04. 240 hens were allotted to a treatment group while 240 hens were allotted to a control group (in accordance with proposed statistical parameters. The study continues to proceed according to original proposed time-lines.
- To date: Approximately 70 hens remain in each group. Tissue samples from expired hens have been harvested and placed in formalin with refrigeration. Histologic examination will be performed following the sacrifice of remainig hens currently planned in 10/06. Differences in incidence of gential tract adenocarcinoma will be determined histologically and reported.

REPORTABLE OUTCOMES.

While reports of results from this study are awaiting final determination of cancer incidence in the laying hens, the work from this study has supported successful funding in similar areas investigating the ability of NSAIDs to prevent ovarian adenocarcinoma in animal models of ovarian cancer. Specifically, an award has been received by Mack N. Barnes MD (PI) from the University of Alabama SPORE in Ovarian Cancer (Grant #P50 CA83591) to examine the ability of the NSAID, Indomethacin to prevent the development of ovarian carcinoma in a murine model. Specifically, this study investigated the ability of indomethacin to inihibit the spontaneous development of ovarian carcinoma as described by Dinelescu et al (5). Specifically, mice with activated K-ras are crossed with mice that have a PTEN gene flanked by stretches of DNA targeted by recombinase. Following injection of Cre recombinase construct into the ovarian bursa of these mice, with resultant expression of K-ras and inactivation of PTEN, metastatic endometroid ovarian adenocarcinoma is observed. These invasive endometrioid adenocarcinomas of the ovary typically arise within 7-12 weeks after injection. In this pilot study a cohort of mice exposed to indomethacin will be compared to control mice not exposed.

CONCLUSIONS

Final conclusions regarding the ability of a COX-2 inhibitor to prevent the development of genital tract adenocarcinoma awaits histologic analysis of all tissue specimens. We anticipate that all specimens will be harvested by the end of 10/06 and pathologic examination can commence. These results will be reported in the final submission to DOD. The project as a whole continues on the originally proposed timeline with work/tasks completed on schedule. *So what section:* New paradigms toward reducing deaths attributable to ovarian cancer

are desperately needed. Due to lack of specific symptoms associated with early disease and absence of accurate screening tests, chemoprevention remains a viable investigational strategy. Demonstration of a reduced incidence of genital tract adenocarcinoma in the laying hen model, would offer significant support for clinical trials directed toward the development of chemoprevention strategies using NSAIDs in patients at elevated risk of ovarian cancer development.

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APPENDICES

None at present

SUPPORTNIG DATA

Please reference tables included in body. Complete tables and figures will be provided in the next submitted report as this document will contain information obtained from histologic examination of all hens that will be performed beginning in October of 2006.